REMARKS/ARGUMENTS

The Official Action and the cited references have been carefully reviewed. The review indicates that the claims, especially as presently amended, recite patentable subject matter and should be allowed. Reconsideration and allowance are therefore respectfully requested.

In advance of addressing the grounds upon which the rejections have been made, a brief summarization of the essentials of the invention process will be provided to:

1) enable ease of grasp of the advance made to the art by providing a method of non-destructive, external or remote, on-line way to monitor the physiological state of stressed (nutrient deprived stress and sulfur deprived stress) of oxygenic photosynthetic microorganisms having a hydrogenase in-situ with regard to their ability to produce H_2 gas; and

2) establish a clearer line of distinction between the invention process and those processes disclosed in the combination of Ghirardi and Melis taken with Seibert.

Applicants are the first to invent an in-situ fluorescence method for external on-line monitoring of the physiological state of a sulfur-deprived algal culture inside a closed photobioreactor system containing an oxygenic photosynthetic microorganism having a hydrogenase in-situ in regard to its ability to produce H_2 gas without relying on electrodes or other sensors placed in direct contact with the culture medium.

This has unexpectedly been accomplished by:

a) providing a sample of sulfur-deprived algal culture containing photosynthetic components;
b) illuminating said sample with artificial or natural illumination;
c) determining the onset of H_2 photoproduction by measuring the percentage of H_2 in a produced gas phase at multiple times to ascertain the point immediately after the anerobiosis subsequent to the physiological phases of O_2 production

and O_2 consumption sequence to obtain data of H_2 production as a function of time; and

d) determining any abrupt change in the following three *in situ* fluorescence parameters:

i) an abrupt increase in F_t (the steady-state level of chlorophyll fluorescence in light adapted cells);

ii) an abrupt decrease in F_m' (the maximal saturating light induced fluorescence level in light adapted cells); and

iii) a precipitous and abrupt decrease in $\Delta F/F_m' = (F_m'-F_t)/F_m'$ where $\Delta F = F_m' - F_t$ (the calculated photochemical activity of photosystem II (PSII)) that signals the full reduction of the plastoquinone pool between PSII and PSI, which indicates the start of anaerobic conditions that in turn induces the synthesis of the hydrogenase enzyme required for subsequent H_2 production, and thereafter slowing down of the abrupt decrease and partial recovery of $\Delta F/F_m'$ signals at least partial oxidation of the plastoquinone pool as the main factor to regulate H_2 production under sulfur depletion.

Claims 1-16 were rejected as being unpatentable over the combination of each of Ghirardi and Melis in view of Seibert under 35 USC 103(a).

Applicants respectfully traverse this rejection and request reconsideration for reasons hereinafter set forth.

A review of Ghirardi et al. shows that it discloses two approaches to obtaining H_2 from a green renewable source. The first process is one in which photosynthetic O_2 and H_2 gas is produced and spatially separated. In this two-stage process, CO_2 is first fixed into H_2 – rich endogenous substrates during normal oxygenic photo-synthesis (stage 1), followed by light-mediated generation of molecular H_2 when the microalgae are incubated under anaerobic conditions (stage 2).

The second is a process in which photosynthetic O_2 and H_2 gas production occur simultaneously, and electrons that are released upon photosyntheticH$_2$O oxidation feed into a hydrogenase-mediated H_2-evolution process, without involving intermediate CO_2 fixation and energy storage as cellular metabolites.

Clearly, neither Ghirardi nor Melis acknowledges the need for ascertaining a non-destructive, external, on-line monitoring of the physiological state of a sulfur-deprived algal culture inside a closed photobioreactor system to alleviate relying upon electrodes or other sensors placed in direct contact with the culture medium, for purposes of determining the ability to produce H_2 under sulfur depletion. Neither do these references disclose or teach a process for addressing a need for a non-destructive, external on-line monitoring system in a closed photobioreactor.

The deficiencies discussed above in connection with Ghirardi and Melis are not compensated for in any teachings in the secondary reference of Siebert.

Accordingly, even if the system using the sensor of Seibert were substituted into the process of the references of Melis and Ghirardi, applicants' process would not result for the reason that applicant is utilizing a method for ascertaining the physiological state of stressed or sulfur deprived oxygenic photosynthetic microorganisms externally, in a non-destructive manner, which is neither hinted at nor taught in any of the combinations of the references applied.

Withdrawal of the rejection is respectfully requested.

Claims 1-16 were rejected under the second paragraph of 35 USC §112 on allegations of indefiniteness with regard to particularly pointing out and distinctly claiming the subject matter of the invention. However, in view of the amendments made to these claims, it is believed that the rejection is no longer applicable. For example, claim 1 has been amended to recite that the

in-situ fluorescence method is external and performed on the physiological state of a sulfur-deprived algal culture inside a closed photobioreactor system. Claim 1 has also been amended to make apparent the definition of ΔF upon resolution of the algebraic equation.

In view of the foregoing amendments, remarks and arguments, it is believed that the application is now in condition for allowance and early notification of the same is earnestly solicited.

Respectfully submitted,

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